(*NEW SERIES.*)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

ON A PARASITE FOUND IN PERSONS SUFFERING FROM ENLARGEI OF THE SPLEEN IN INDIA, SECOND REPORT.

BY

LIEUT. S. R. CHRISTOPHERS, M.B., I.M.S.
(*On special duty.*)

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF IND BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA.

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s for the Sale of Books published by the Superintendent of Government Printing, India, Calcutta.

In England.

OLD, 37, Bedford Street, Strand, Lon-
: & Co., 2, Whitehall Gardens, London,
; & Son, 2 & 4, Great Smith Street, ster, London, S.W.
UL, Trench, Trübner & Co., Charing ıad, London, W.C.
Quaritch, 15, Piccadilly, London, W. C.
and Norgate, Oxford.
Bell & Co., Cambridge.

On the Continent.

ander & Sohn, 11, Carlstrasse, Berlin,
ıRassowitz, Leipzig.
Hiersemann, Leipzig.
Ernest Leroux, 28, Rue Bonaparte, Paris.
Martinus Nijhoff, The Hague, Holland.

In India.

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Rai Sahib M. Gulab Singh and Sons, Mufid-i-Am Press, Lahore.
N. B. Mathur, Superintendent, Nazair Kanun Hind Press, Allahabad.
Superintendent, American Baptist Mission Press Rangoon.

On a Parasite found in Persons suffering from Enlargement of the Spleen in India, Second Report.

In my first report I recorded briefly the result of the researches of different observers on the parasite recently discovered by Leishman and Donovan in cases of tropical splenomegaly. Such investigations, in many cases of enlarged spleen, have shewn in blood drawn during life from the spleen and liver or in films of these organs *post-mortem* the presence of certain very definite bodies of peculiar nature and uniform morphology. In the case of preparations made during life a matrix-like substance is conspicuous in which many of the bodies lie embedded. The actual nature of the matrix has given rise to much discussion. It is considered by Laveran and Mesnil to be altered red corpuscle, by Ross a parent mass producing the bodies, by Manson and Low it is termed a "zooglea mass." In my last report I gave many reasons for considering the matrix to be largely, if not entirely, the fragmented, budded and vacuolated cytoplasm of cells in which, in sections, the bodies are seen lying.

In all the researches mentioned the attention of the observer had been entirely occupied with the presence of the parasite in the blood.

Wright's announcement that similar or very closely related bodies occurred in large numbers in the tissues of tropical ulcer gave rise therefore to considerable surprise.

The microscopical appearances of tropical ulcer were described by Cunningham in 1885.[1] He notes extensive infiltration of the corium and subcutaneous tissues by granulation tissue containing bodies which he considered to be parasitic in nature. From Cunningham's drawings it would appear that these were macrophages or other cells containing the bodies described by Wright, though Cunningham figures no structure in these latter and terms them nucleoid bodies.

Wright [2] defines tropical ulcer as a single or multiple focal lesion of the skin characterised by the formation of elevated and indurated areas which ulcerate and eventually cicatrise. He draws attention to the resemblance to certain forms of cutaneous tuberculosis and syphilis. He describes, and gives photographs. shewing an extensive infiltration of the corium and subcutaneous tissue with cells. In addition to plasma cells and various kinds of lymphoid cells, he notes large cells with vesicular nuclei, the cytoplasm of which contains numerous peculiar bodies having a very constant morphology and structure. An examination of the excellent photographs of these bodies leaves no doubt as to their

ıorphological identity with the forms seen in cases of enlarged spleen in Madras. Wright describes the bodies as largely occurring in the cytoplasm of ells, especially of the large cells with vesicular nuclei. His photographs shew nat the great majority of the bodies are situated in the cytoplasm of these cells vhich appear identical with those I have already described in the spleen, liver, nd bone-marrow and which I have termed macrophages.

Wright's discovery would appear to modify considerably existing views as to he nature of the parasite, since the presence of the bodies in immense numbers n the cytoplasm of infiltrating cells in a focal skin lesion seems opposed to their upposed rôle as parasites of the red cell or even as solely parasites of the ılood. Laveran and Mesnil,[3] however, still maintain the relation of the parasite o piroplasma. Examining slides sent to them by Donovan, they describe very ımall and ate forms in the peripheral circulation which may be free or may be ıituated i· an unaltered red cell. The small size, the evident great rarity and ·specially the absence of the characteristic double chromatin masses make the elation of these forms to the parasite very doubtful. I have examined both peri-ıheral and splenic blood for appearances which would seem to point to the origin ıf the bo'es in the red cells, but without result.

In m· first paper I drew attention to certain very definite clinical features of his disea vhich is, as a rule, readily diagnosed. I also described three autopsies ınd gave ·.e result of an examination of some of the organs and tissues. In the ıresent r· ·t I shall consider the results arrived at under the following heads

(1· A description of four further autopsies.

(2) The salient and pathological nature of the disease.

(3) The relation of the parasite to the tissues of the host, especially demonstrating a type of infection in which the vascular endothelium is principally implicated.

(4 Certain points in the morphology of the parasite and in the nature of the "matrix" or "zooglea mass."

(5· A comparison of the conditions found *post-mortem* in infection with the new parasite with those found in trypanosomiasis.

Autopsies in fatal cases of the disease.

AUTOPSY 4.--Child aged about 12. Emaciation marked. Death from *cancrum oris*.

Spleen much enlarged, of firm consistence and dark red in colour, but not pigmented.

Liver considerably enlarged; very pale and mottled in appearance. On section the mottling was seen to be due to the presence of new tissue of pale hue replacing the centre of the lobule and extending about half way to the periphery.

The line of demarcation between the pale tissue and the darker liver tissue was very distinct. Microscopical examination shewed that the above appearances were due to the fact that in the centre of the lobule the liver cells were markedly atrophied, and the liver substance almost replaced by the large cells described in the last report.

Intestines.—The mucous membrane was pale, but otherwise quite normal in appearance.

The lymphatic glands behind the mesentery were about the size of beans and of normal appearance.

A large hæmorrhage was present under the peritoneum of the under surface of the diaphragm extending over several square inches. Several smaller hæmorrhages were present in the neighbourhood. Prior to death the spleen had not been punctured.

The œsophagus was examined closely with a lens but no unusual appearances were seen in the muscular coats.

The muscles were examined in several situations with a lens but shewed no unusual appearances.

The heart, kidneys, pancreas, suprarenals and *bladder* were normal in appearance.

The lungs shewed areas of congestion and there were small subpleural hæmorrhages over the bases.

Distribution of the bodies.—Bodies were present in large numbers in the spleen, liver and bone-marrow. Sections of the liver and spleen shewed, as in the tissues of the first three autopsies, many large cells crowded with the bodies lying in the capillaries. In the spleen large branched cells which appeared to assist in forming the splenic reticulum also contained many bodies in their cytoplasm. In blood from the hepatic veins bodies were seen in cells of mononuclear and endothelial type as well as in polymorphonuclear cells. In blood taken from a small vessel in the muscles of the thigh a fair number of bodies were present in large mononuclear cells.

Various connective tissues were closely examined for the parasites but without result.

Muscle spread upon the slide by the "half-drying method" and stained by the modification of Romanowski described in the first report did not reveal any bodies.

AUTOPSY 5.—Man aged about 40. Fairly well nourished. Death from peritonitis.

Abdomen contained about two pints of purulent fluid which was collected especially in the neighbourhood of the cæcum.

Spleen somewhat enlarged. There were two large infarcts and many smaller ones visible.

Liver.—Nearly one half of the liver was light yellow in colour and necrotic in appearance. The condition appeared to be an enormous infarct. The remaining liver substance shewed, to a less degree, the lobular changes described in case 4. An abscess cavity containing necrotic tissue and about the size of a small orange was situated in the non-infarcted liver tissue.

Small intestine normal in the greater portion of its length. Peyer's patches in the lower few feet were prominent.

Large intestine except the cæcum shewed no marked changes. The cæcum over an area of several square inches was gangrenous and perforation at one point had taken place. The appendix was normal.

Stomach. –There were several extensive areas over which the mucous membrane was infiltrated with blood, and of a gelatinous consistence. Small areas congestion were present in the neighbourhood of these areas.

Œsophagus.—The lower two inches of the œsophagus was occupied by a large sloughing ulcer which had completely destroyed the mucous membrane.

Kidneys were pale and shewed fatty degeneration of the convoluted tubules.

Pancreas was soft and flabby.

Heart.—The valves were normal. The endocardium was stained red.

Lungs.—There was marked congestion of both bases.

Brain.—Normal.

Skin.—On the skin of the front of the right thigh there was a mark resembling the cicatrix of a healed ulcer. On incision a deeply situated caseous mass, the size of a large pea, was disclosed surrounded by red granulation tissue and dense fibrosis. The skin over the caseous mass was thin and on the point of absorption, the appearance of a cicatrix being deceptive.

On the skin of the thighs numerous small pale areas could be detected apparently of the nature of cicatrices after superficial ulceration.

Distribution of the bodies.—Large numbers of the bodies were present in the spleen, liver and bone-marrow. Many single bodies were seen lying in cells in the granulation tissue around the caseous nodule in the skin. In the small vessels in the immediate neighbourhood of this tissue many large cells crowded with the parasites were seen. Sections of the normal skin of the thigh shewed no bodies, nor were the characteristic large cells seen in the vessels. A lymphatic gland in the right groin shewed numerous bodies in large cells in the lymph sinuses and in the stroma cells of the lymphoid tissue. In certain areas of the testis many single bodies were seen and large cells containing many bodies were seen in some of the small vessels. A few cells containing a number of bodies were found in films from the kidney substance and from the lung. Sections of the kidney did not reveal any parasites. In blood from the femoral vein bodies were found included in leucocytes and endothelial cells. In the films of different organs and in the blood many large

encapsuled bacilli were seen in leucocytes. In the capillaries of the brain some of the endothelium cells were packed with this organism. Some of these *post-mortem* appearances would appear to have been due to a secondary bacterial infection.

AUTOPSY 6.—Man aged about 45. Cause of death, large meningeal hæmorrhage.

Spleen large, firm, dark red, and not pigmented

Liver large, pale and mottled. The lobules shewed the changes described in case 4.

Small intestine.—Normal.

Large intestine.—Numerous small ulcers extending to the muscular coats were scattered over the whole length of the gut. There were several raised plaques formed by papillomatous-like outgrowths. On microscopical examination the projecting mass was seen to be granulation tissue, and the crypts of Lieberkuhn in the neighbourhood were infiltrated with new tissue and in process of destruction.

Brain.—Under the dura mater of the left hemisphere there was a large clot covering the greater part of the cortex and occupying the space at the base of the brain. There was no fracture and no extravasation of blood outside the dura mater nor in the tissues of the scalp. There was no blood in the ventricles. The brain substance appeared normal.

The larger arteries were free from atheroma.

The heart, lungs, pancreas and *kidneys* appeared normal.

The skin.—There were no skin lesions.

The distribution of the parasite.—Bodies were present in large numbers in the spleen, liver and bone-marrow. They were not detected in an inguinal lymphatic gland nor in the skin. The distribution of the bodies in the other organs has not yet been worked out.

AUTOPSY 7.—Girl aged about 16. In hospital for large sloughing ulcer of the foot. Cause of death—*noma* of the vulva.

Spleen large, firm, dark, and not pigmented.

Liver large, pale and mottled. Lobular changes as in case 4, very marked.

Small intestine.—Normal.

Large intestine.—There were many depressed and pigmented areas scattered over the whole of the mucous membrane of the large bowel. Except that the ulcers had healed, the condition was very similar to that found in autopsy 6.

Skin.—On the inner side of the left heel was an ulcer about the size of a rupee. On the dorsum of the right foot was an ulcer the size of the palm of the hand, exposing the deep tissues and bones. The vulva was in a sloughy and very foul condition.

Distribution of the parasite.—Bodies were very abundant in the liver and spleen. The bone-marrow was not examined. In both ulcers single bodies were seen lying in the organised granulation tissue in the bases and also in the healthy tissues beyond. Large cells crowded with the bodies were seen here and there in the small vessels in the neighbourhood of the ulcers.

GENERAL *POST-MORTEM* APPEARANCES.—It will be seen from the seven autopsies now described that there are certain constant pathological appearances associated with infection by the new parasite. We may summarise these as follows.

The *post-mortem* appearances of the liver and spleen are in themselves almost pathognomonic. The spleen has a peculiar smooth, firm and solid look, and it retains its shape after removal from the body like an organ hardened *in situ*. The substance is dark red, granular and homogeneous. The trabeculæ shew up clearly against the dark pulp. The consistence is firm but friable and the sensation given to the finger is quite different to that given by a tough fibroid spleen. The liver retains its shape after removal. The liver substance is firm but friable. The surface is mottled, suggesting the nutmeg liver of passive engorgement, but the general colour is lighter instead of darker than normal. On section, the mottled look is seen to be due to a white tissue which occupies the centre of the lobules and gives rise to an arborescent appearance. Microscopical examination shews that the white tissue is a new deposit formed of macrophages and their contained bodies, which have practically replaced the liver tissue of the centre of the lobule.

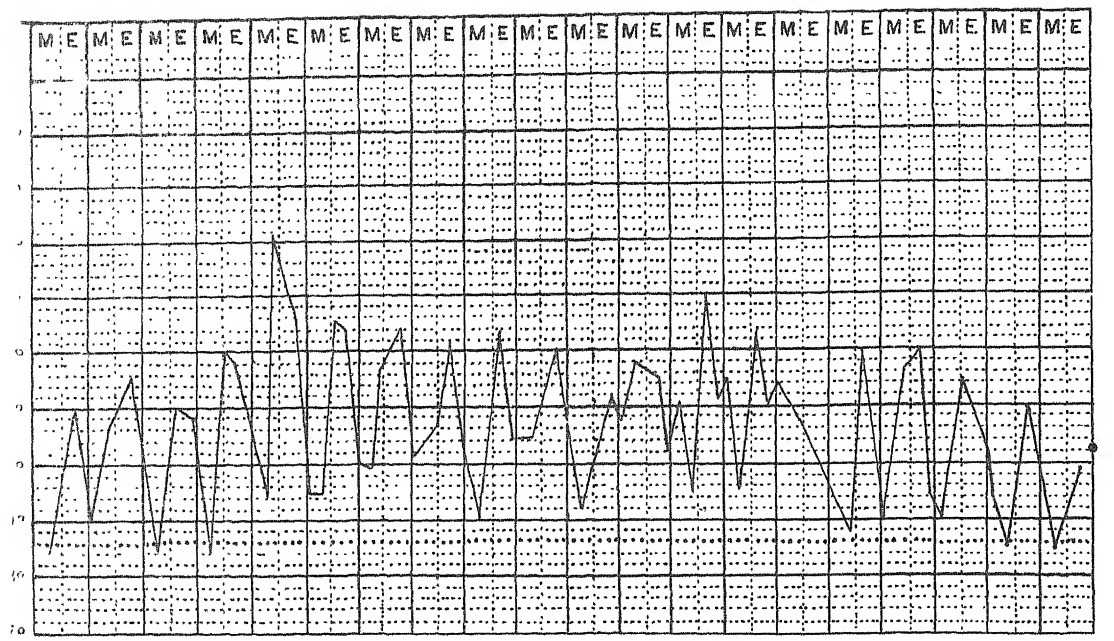
The large intestine shews, almost constantly, extensive multiple ulcers which are deep and sloughy and tend to perforate the muscular coats. The occurrence of fungating granulation tissue which has a papillomatous appearance is seen in association with the ulcers.

Purulent peritonitis and bronchopneumonia are frequent, arising, respectively, from the perforation of intestinal ulcers and as a sequel to *cancrum oris*.

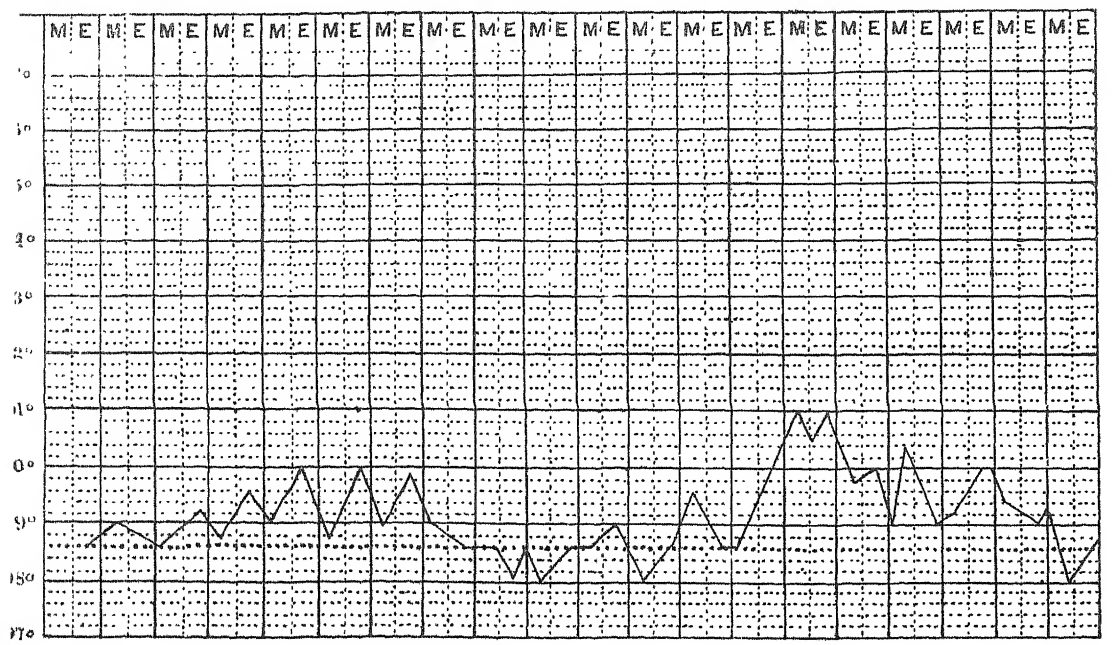
Septic conditions associated with the formation of infarcts in the organs are sometimes superadded.

The small intestine rarely shews any lesions. The heart, kidneys, brain, gall-bladder, urinary bladder, pancreas, suprarenals, testicles, lymphatic glands and muscles shew, as a rule, no macroscopic changes.

The parasites are found constantly in immense numbers in the cells of the liver, spleen and bone-marrow, in granulation tissue in the intestine and in the skin. They were present in large numbers in a lymphatic gland draining an area in which a skin lesion was present, but not in glands where no such condition existed. Bodies are, in some cases at least, fairly numerous in patches in the testicle. In the kidneys they do not appear to be numerous. They are present in some



ASE 1.—Advanced case of the disease. Spleen reaching to umbilicus. Papules and small ulcers. Diarrhœa.



CASE 2.—Well developed disease. Spleen well below the costal margin.

cases in the lungs. In the blood of the large veins they appear to be fairly numerous included in various types of cells.

The Clinical and Pathological nature of the disease.

In the first report I drew attention to certain very constant clinical features in cases of systemic infection by the parasite. These were—

(1) A great enlargement of the spleen.
(2) Emaciation.
(3) An irregular high temperature.
(4) Abdominal symptoms.

I have since been able to add the following which appear to be equally characteristic.

(5) A tendency to *noma* and local gangrenes.
(6) The frequent presence of a papular eruption and small or large ulcers of the skin.
(7) The occurrence of hæmorrhages.

Enlarged spleen.—Enlargement of this organ has been very constant. In Madras, bodies are almost always found on puncture in spleens which are so enlarged as to project several inches below the costal margin. On the other hand, in a few cases the spleen has been only moderately enlarged. In two cases there was a high temperature and severe constitutional disturbance associated with considerable enlargement of the spleen. Bodies were found, but were extremely scanty in both cases.

Emaciation.—Although emaciation is generally present, it is not invariably so, and death may occur from several causes connected with the disease without wasting being a prominent sign.

Temperature.—An irregular temperature has been noted as a feature of the disease. This is almost always present in the final stages. But even when splenic enlargement is considerable and emaciation marked, a high temperature may be absent, or slight rises only above the normal may occur. The most frequent type of temperature is that shewing irregular rises to 102° or 103° F.; at times a temperature of 104° or even 105° is reached. The two charts which accompany this report, for which I am indebted to Major Robertson, I.M.S., are typical of the disease as most frequently seen. There appears to be an acute form of the disease. In two cases in which the spleen reached half way to the umbilicus there was high fever which afterwards fell to normal as in enteric fever. In both cases it was very noticeable that the bodies were present in very small numbers in blood drawn from the spleen. Major Robertson, I.M.S., informs me that such cases are frequently readmitted to hospital with similar attacks.

Abdominal symptoms.—The constancy with which abdominal symptoms supervene has been already commented upon. These were shewn in the first report to depend upon the formation of extensive ulcers in the large gut. Of the seven autopsies, in five there was ulceration of the large intestine, and in one gangrene of the cæcum. In one case only was the mucous membrane of the large intestine normal. The dangerous nature of intestinal conditions is well shewn in the autopsies recorded, where three out of seven deaths were directly due to perforation of the gut. Healing of the ulcers appears to take place, since in one case, only numerous pigmented and depressed scars were present

In the first report it was shown that the above lesions were associated with the presence of the parasites, often in very large numbers. In autopsy 6, single bodies were found scattered through the organised granulation tissue, and in the young tissue about the crypts, large cells were found as in autopsy 3. Bodies which appeared to be multiple division forms appeared more common in the granulation tissue from the intestine of case 3 than in the spleen, but the rapid changes in the tissues of the intestine leading to fragmentation of the nuclei render it difficult to be sure of such forms.

The amœba coli is by no means uncommon and is found in the granulation tissue in the intestines and in the walls of abcesses in the liver. How far the intestinal condition is due to the presence of this organism it is impossible at present to say. Amœbæ were found in cases 2, 3 and 5. Small amœbæ with a single chromatin mass were seen in the intestine as well as large forms in which the chromatin mass has undergone division. In the liver abscesses large circular forms were seen. The intestinal forms were identical with those seen in a typical case of severe dysentery in a European. I have not been able after long search to find any forms transitional between the amœba and the parasites under discussion. In two deaths from typical dysentery in which the amœba coli was present, the new parasite was not found in the spleen.

The occurrence of noma and local gangrenes.—Of the seven cases recorded, two died of *cancrum oris*, one of *noma* of the vulva, and one of gangrene of the cæcum. In two of these cases emaciation was not present and the body appeared well nourished. In one case there was, in addition to the *noma*, a large ulcer of phagedenic type which had exposed the bones of the foot.

Noma undoubtedly plays a most important part in bringing about death from this disease. *Noma* and perforation of the large intestine would appear indeed from our cases to be the chief causes of death in infection by the parasite.

Skin eruption and the occurrence of ulcers: Ulcers.—Shortly after the discovery by Wright of the bodies in tropical ulcer, Major Donovan,[6] reported the presence of the parasite in small ulcers which are often found about the knees

and elbows of cases of the disease. I have examined scrapings and small pieces of tissue snipped from such ulcers in three advanced cases of the disease and have found bodies in small numbers in each case. The ulcers usually seen are small, from 2 to 10 *m m.* in diameter. They are covered with a thick raised scab and the base of the ulcer is usually depressed and devoid of granulations The edges are usually thickened. The origin of the ulcers is doubtful. I have always failed to detect sarcoptes in them and they appear to arise from ulceration of a papular eruption which is generally found along with them.

In some cases larger ulcers are seen about the legs. They vary from the size of a shilling to extensive raw surfaces several inches in diameter. In autopsy 7, ulcers of this nature in section shewed scattered bodies throughout the tissues of their bases, and in the healthy skin in the immediate neighbourhood Very scanty bodies were also seen in snippings taken from such ulcers in a similar case (Pl. II, fig. 12).

In the granulation tissue surrounding the caseous mass in the dermis of case 5, bodies were present in considerable numbers, and large numbers of bodies were found in sections of a femoral gland above this lesion.

I have examined a number of large and small ulcers in the out-patient department, but so far have never detected bodies where there was no general infection with the new parasite.

Papular eruption.—It is common in cases of the disease especially in the advanced stages to find a scanty or profuse papular eruption about the thighs, Scarpa's triangle, and the scrotum. Similar papules are less frequently seen on the trunk, arms and neck. Some of these appear to ulcerate and form small ulcers covered with a raised scab, which are evidently slow to heal and chronic in nature. An unulcerated papule from a case shewed, in section, bodies in small numbers scattered through the dermis. Since we have been unable to find bodies in normal skin, it is possible that the eruption is an integral part of the disease. It appears distinct from the scabies seen on the hands of many of the cases, and the papule examined shewed a deeper lesion than in this condition.

With regard to the skin lesions in general I am unable to say whether they are due to the disease or other causes. The lesions have no very marked characters by which they may be detected or differentiated from those occurring in natives not suffering from the disease. On the other hand, the presence of the parasites in them is important.

Hæmorrhages.—Small petechial hæmorrhages are of fairly constant occurrence in fatal cases. They are especially frequent in the serous membranes, peritoneum, pleura and meninges. Larger extravasations of blood in similar positions and into the mucous membranes appear also to be not infrequent. In one case death occurred from a large hæmorrhage under the dura mater.

The relation of the Parasite to the tissues of the Host.

Skin - [illegible] papule was removed from the thigh of an advanced [illegible]. The tissue was fixed in absolute alcohol, embedded in paraffin [illegible] by the modification of Romanowski noted in the [illegible]. In sections cut through the deeper parts of the corium a layer of [illegible] which refused to take on the Romanowski stain [illegible]. Scattered throughout the dermis were isolated [illegible]. There were rarely more than three or four to be seen in any [illegible]. They occurred close beneath the epithelium in [illegible] papule and to the full depth of the tissue removed (about 2 *m m.*). [illegible] appeared to have no relation to the blood vessels and to [illegible]. Close examination showed that such bodies were in [illegible] which appeared to be endothelial in nature [illegible] of very fine dermal capillaries. (Pl. I. [illegible]). In most cases the bodies were not surrounded by a [illegible] a delicate capsule was present.

[illegible] in glycerine from autopsy were fixed in absolute alcohol [illegible] and were treated with Romanowski's stain. The base of [illegible] to the depth of 2 *m m.* was formed of vascular tissue largely composed [illegible]. Such parasites were seen in the endothelium of [illegible]

[illegible] Leishman's stain. [illegible] the tissue [illegible] and treated [illegible] of Leishman's stain [illegible] stained more pale pink, [illegible] the tissue [illegible] to [illegible] the tissue [illegible] the side and [illegible] Leishman's stain [illegible] Leishman's stain [illegible] the tissue as usual with xylol, [illegible] (Gruber).

[illegible] with distilled water, [illegible] with an excess [illegible] and allow to stand for 15–30 minutes [illegible] the tissue moving by tilting the slide as [illegible]

[illegible] distilled water

[illegible] sodium carbonate

[illegible] Leishman's stain

[illegible]

Wash out the stain with distilled water, and blot at once after washing in distilled water. Repeat the washing and blotting until the tissue becomes free from blue stain and the nuclei are a clear bright red.

Allow the section to dry upon the slide. Mount in Canada balsam or allow to remain uncovered as in the case of a film. Sections mounted in Canada balsam last two or three months but eventually lose their colour.

The method is applicable also to tissues such as areolar tissue or muscle which can be spread upon a slide by the ordinary manner.

the capillaries throughout the whole area of the [illegible] beneath the ulcerated epidermis. In the sections there were a few capillaries large cells containing from 6 to 12 bodies were readily seen.

3. The edges of the ulcerated nodule and the surrounding [illegible] autopsy 5 were fixed in absolute alcohol, cut in paraffine and stained with Romanowski's stain. The central ulcerated mass was composed of necrotic cells of several varieties, chiefly small lymphoid cells and large mononuclear cells. A certain number of polymorphonuclear cells were present but they did not form the great majority of cells as in ordinary pus. The necrotic tissue was surrounded by granulation tissue which extended to a distance of a few millimetres between the layers of dense fibrous tissue, which surrounded the whole. Bodies were found readily in the granulation tissue, and wherever an infiltration of healthy tissues with cells was taking place. As many as ten, and in some cases more bodies were to be seen in each field of the microscope. In the young granulation tissue composed of lymphoid cells and larger mononuclear cells the parasites were seen in every case lying embedded singly in the latter type of cell. In the capillaries in the neighbourhood, bodies were seen lying singly, or in twos and threes, in the endothelium cells. In the smaller vessels in the neighbourhood of the granulation tissue large cells containing numerous parasites were seen. These were similar to the cells seen in the liver and spleen. They were most often attached by one side to the vessel wall whilst the mass of the cell projected into the lumen of the vessel (Pl. II. fig. 9). In the somewhat larger vessels they were often much more free in the lumen. In a sweat gland which had been invaded by the granulation tissue, many cells, each containing several bodies, were seen. These lay in close connection with the coils of the gland apparently in fine capillaries which surrounded the coil. In adipose tissue which had been invaded by granulation tissue, single bodies lying in the endothelium cells of capillaries were readily seen. In parts of the section remote from the granulation tissue bodies could not be found either in the capillaries or larger vessels. In the capillaries of healthy adipose tissue no bodies were to be made out. In these sections all stages between unaltered endothelium cells containing a single body, and large cells partially detached from the vessel wall and containing many bodies, could be made out.

4. Sections of apparently normal skin from the thigh of the same case were carefully searched, but no bodies were found. In the neighbourhood of the pale cicatrix-like areas already noted, many large cells loaded with pigment of the same nature as the epithelial pigment were seen in the tissues and occasionally in the smaller vessels.

5. Areolar tissue from the skin and subperitoneal tissue were spread upon slides and stained by the Romanowski method. The tissue elements were well displayed, but no bodies were seen.

[illegible] from [illegible] apart from general infection [illegible] In sections [illegible] lying upon the skin, large cells with [illegible] and [illegible] resembling the smaller macrophages, are seen [illegible] with [illegible] a greenish pigment which appears to be derived from the skin.

In sections of the axillary lymphatic gland taken from the right groin [illegible] and stained by Romanowski's method showed, in the lymph [illegible] and [illegible] of lymphoid tissue, many large cells [illegible] the parasite bodies. Similar cells containing pigment of the same [illegible] as that seen in sections of the skin were also present in the same position, and [illegible] cells contained both pigment and parasites (Pl. I. fig. 6). Many [illegible] showed the necrotic changes and central vacuolisation to be [illegible] (Pl. I. fig. 6). In some cases they appeared to be on the point of [illegible] Many of the [illegible] had the cortex and medulla, but especially the former, crowded with groups of the bodies (Pl. I. fig. 5). Sections of a [illegible] from [illegible] in which no skin lesion was present, showed no bodies.

In the [illegible] Very thin sections of the intestine in autopsies 3 and 6 showed [illegible] in the [illegible] granulation tissue. In almost every case [illegible] in clear vacuoles which appeared to be the cytoplasm of [illegible] cells of the [illegible]. In the younger granulation tissue, and especially in [illegible] in the crypts of Lieberkühn, the bodies were seen in [illegible] mononuclear [illegible] cells, in [illegible] groups. The number of bodies [illegible] from the same area varied very much.

[illegible] sections of the testis from autopsy 3 showed among the tubules [illegible] a [illegible] granular deposit. On closer examination [illegible] were seen to be [illegible] of endothelioid cells. In these areas [illegible] were present in considerable numbers lying in the cytoplasm of the cells. In some of the [illegible] cells were seen containing [illegible] (Pl. I. fig. 4), and in the still larger vessels crowded cells were present. The bodies were not present in the capillaries in all parts of the testis.

LUNG.—In autopsies 4 and 6 fairly numerous large crowded cells were seen in [illegible].

KIDNEY.—Films from autopsy 6 showed occasional cells of endothelial nature containing from 5 to 20 bodies. Only a small piece of kidney cortex was reserved for sections and no bodies could be seen in this. The glomerular capillaries were carefully searched but in vain. Sections of the cortex and medulla of the kidney from case 1 were examined but no bodies were found.

LARGE VEINS.—Blood films made from the large veins, femoral, portal, hepatic, some hours after death, showed cells containing parasites which were first thought to be peculiar forms of large mononuclear leucocytes. It seems certain that

these cells are in reality endothelial in nature, since they resemble closely cells seen forming the walls of small vessels exposed in longitudinal sections. I have thought it possible that they may become detached from the vessel wall after death (Pl. II. fig. 6 and fig. 7).

SPLEEN AND LIVER.—In blood obtained from these organs the parasites are out of the parasites are seen either free or included in a vacuole. In sections of the organs no such relation is to be made out and the parasites are seen lying in the cytoplasm of cells. The cells are of two kinds.

Leucocytes.—Laveran and Mesnil, Ross and Manson and Low all call attention to the existence of phagocytosis and the presence of bodies in the large mononuclear and polymorphonuclear leucocytes. In my first report I have also considered the part played by the leucocytes in taking up the parasites.

The endothelium.—Marchand and Ledingham note the occurrence of the parasite in very large cells in the spleen and bone-marrow. In my first report I also made mention of these enormous cells, which appear to be of a similar nature to the macrophages seen in fatal cases of malaria. The careful study of sections of the spleen and liver in cases of infection with the new parasite has led me to conclude that the macrophages are derived from the vascular endothelium and that they represent a final stage of endothelium cells which have become more and more modified and distended with included parasites. It is indeed easy to trace every gradation between flattened endothelium cells containing a few bodies and the enormous swollen cells almost blocking the capillary in which they lie. In both the liver and spleen, many more bodies are included in cells of endothelial nature than in the leucocytes. In films this fact is masked by the rupture of many of the more distended endothelial cells, and also by the fact that many endothelium cells approximate in appearance to cells of large mononuclear leucocyte type and may be readily classed in this group. Two cells, in reality endothelial in nature, are figured by me as large mononuclear leucocytes in my first report (fig. 22) and it is possible that others have erred similarly. The following types of endothelium cell in which the parasite occurs may be noted.

1. Endothelium cells but little modified. In films these have round, oval or kidney-shaped nuclei and extensive protoplasm which is often arranged so as to give the cells an elongated appearance. The protoplasm, as a rule, shows a tendency to vacuolisation especially towards the free ends of the cells (Pl. II. fig. 6). It also tends to become protruded in the form of buds. In sections they may be quite flat or they may shew at one, or more rarely both ends, a swollen appearance. They are applied closely to the capillary wall or stroma. They contain, as a rule, from 6 to 12 bodies. Identical cells are seen in the capillaries of the testis and of granulation tissue (Pl. II. fig. 8).

1. Large vacuolated cells with extensive protoplasm and a round or oval nucleus. Small specimens of these cells may give rise to a puzzling appearance in which stage they resemble at first sight the nucleus plus a small monomuclear leucocyte. The protoplasm is, however, much more extensive than is the case with the leucocyte. Here large cells of this type are often seen whole or only part of the protoplasm is lost. Their protoplasm is often like ground-glass in appearance and is usually vacuolated. Most of the protoplasm of these cells may sometimes be separated from the cell (Pl. II. fig. 4), and may assume a globular shape. They contain often six or more bodies. In sections of the testis and of granulomatous tissue, cells which appear to be of this type are seen in the smaller vessels attached as a rule by one side and projecting freely into the lumen. Similar cells are seen in blood taken *ante mortem* from the large veins, femoral, portal, hepatic. (Pl. II. figs. 7 and 9.)

2. In both the liver and spleen, numerous numbers of very large cells are seen lining the capillaries. They may be extended along the capillary, or their protoplasm may be massed together. In the spleen, large cells are seen with long processes extending in among the smaller cells of the pulp. Both types of cell appear to be of the same nature. They have a single or double vesicular nucleus, and their cytoplasm, which stains lightly, has numerous included bodies lying either scattered more or less evenly throughout, or in groups.

4. In both the liver and spleen, but especially in the latter, extremely large cells are seen in which the nucleus is small, dense, centrally placed and is pushed to one side or extremity of the cell. The protoplasm of these cells stains more darkly than in the last mentioned variety, and in some cases takes on a curious hyaline appearance resembling erythrocytes. The centre of these cells is occupied by a large vacuolated space and arranged around this are numerous parasitic bodies. The cytoplasm of many of these cells is ruptured or is more pellicle containing a number of parasites, and such cells appear to be on the point of rupture. Cells of this and the last mentioned type are rarely seen whole in films unless special precautions are taken by fixing in 5 per cent. formic acid. One such cell in the liver measured contained as many as 250 bodies (Pl. II. figs. 10 and 11), another (Pl. I. fig. 6).

Peripheral blood.—Laveran and Mesnil record the presence of bodies in the peripheral blood which they take to be endoglobular forms of the parasite. Such forms, they say, are very rare, smaller than those found in the spleen and have only one chromatin mass. I have been unable to satisfy myself that undoubted bodies occur in the peripheral blood, free or in the red cell. Taking into account the negative results of Manson and Low who examined a case with this object very systematically and my own results on a number of cases, it does not appear that typical forms are present. Laveran and Mesnil's forms do not appear

to be very convincing, and even these must be of extreme rarity. In two advanced cases approaching a fatal termination, I have found in the peripheral blood a considerable number of typical forms presenting double chromatin masses and in every way resembling those found in the spleen, but these were all included either in polymorphonuclear or mononuclear leucocytes. In one case 37 parasites, nearly all in polymorphonuclear leucocytes, were met during a count of 500 leucocytes. In the other case, 7 parasites were seen while counting a similar number of leucocytes. In both cases leucocytes were in excess of the number usually found in the disease in which, as a rule, the most marked leucopenia is found. In neither case did I find at this time free forms or forms in the red cells. In several instances I have examined very carefully films of spleen blood with a view to finding transitional forms between the bodies lying in a matrix and those in unchanged cells, but unsuccessfully. I am led, then, to doubt very greatly the specific nature of the bodies in the red cells.

In the most severe infections the relative leucocyte values do not appear to show very great changes. The leucopenia is the most marked change, and this is, as a rule, so great that it is necessary to take several large films in order to make adequate leucocyte counts. Examples of the values obtained are the following:

Case 1.—Total leucocytes counted 500.

Polymorphonuclear leucocytes	69 per cent.
Large mononuclear "	11 "
Small "	14 "
Eosinophyl cells	1 "
Myelocytes	3 "
Transitional	1 "
Intermediate	1 "

Case 2.—Total leucocytes counted, 500.

Polymorphonuclear leucocytes	69·8 per cent.
Large mononuclear "	6·6 "
Small "	20·2 "
Eosinophyl cells	1 "
Myelocytes	2 "
Transitional	0·4 "
Intermediate	0·0 "

Case 3.—Leucocytes 250 per c. m. m. Total number counted 500.

Polymorphonuclear leucocytes	56 per cent.
Large mononuclear	10·6 "
Small "	29·2 "
Eosinophyl cells	3·6 "
Myelocytes	0·6 "

Case 4.—Leucocytes 437 per c. m. m. Total number counted 500.

Polymorphonuclear leucocytes	75 per cent.
Large mononuclear "	7 "
Small "	15 "
Eosinophyl cells	4 "

[illegible]

With regard to the part played by the spleen and liver it is as yet premature to form conclusions. On the whole it is possible that autoinfection of the spleen and hepatic endothelium is constantly going on and forms the chief process in the disease. The fact that bodies are found in the skin, intestine, lungs and testis as well as in the large veins and in the lymphatic glands, draining certain lesions, shews that wide dissemination of the parasite through the body also occurs, and the function of the spleen and liver as storing organs must not be overlooked.

It is of course possible that the endothelium infection is derived primarily from very rare forms living in the red cells, but it seems, in the present state of

our knowledge, much more probable that the final disintegration of large mononuclear cells is the means by which fresh swarms of the bodies are set free to be taken up again by the endothelial cells of the spleen and elsewhere.

A consideration of the main features of the process here exemplified, namely, an endothelial infection, invites comparison with the processes in certain other diseases.

DISEASES OF SEPTICÆMIC TYPE.—Infection by the new parasite as shown in so-called malarial cachexia has a strong resemblance to certain chronic septicæmias, notably to some forms of malignant endocarditis.

That destruction of red cells is not necessarily due to actual invasion of the corpuscle by the parasite is shown in those cases of septicæmia where the destruction of red cells is usually very great.

The finding of organisms in the circulating blood of cases of septicæmia and pyæmia is also generally difficult and necessitates the use of considerable quantities of blood, a point which has been elucidated especially by Kuhnau.[1] The experiments of Werigo[2] and others indeed shew that it is the endothelium and leucocytes in the visceral capillaries which are mainly implicated when an infection of the blood stream by micro-organisms takes place. We may indeed almost consider this form of splenomegaly as a septicæmia in which the new parasite is the infecting organism. We do not yet know how the parasite enters the blood stream or whether it may give rise to conditions other than those found in splenomegaly or in tropical ulcer, but in these we appear to have two very distinct types of infection.

(1) A local lesion—Tropical ulcer.

(2) A septicæmia—the so-called malarial cachexia of India.

The fact that an infection may be mainly or entirely endothelial appears to me to be important. The examination of the peripheral blood in Indian cachexia is scarcely likely to have led to the discovery of the parasite. In Malta fever splenic puncture is generally necessary in order to detect the infecting micro-organism. Even in pronounced septicæmias, where cultural methods can be employed, the detection of forms in the blood is uncertain, and a negative result generally recognised as being without significance. By puncturing the spleen one removes many endothelial cells and leucocytes which have remained in the visceral capillaries, thus tapping an entirely different tissue from the blood. Such a means of investigation should be a fruitful one in diseases where a blood infection appears probable, but where no organism is to be detected in the peripheral blood, *e.g.*, yellow fever.

DISEASES OF GRANULOMATOUS TYPE.—Tropical ulcer has many features which would lead to its classification as a granulomatous lesion. Both Cunningham and Wright shew that extensive deposits of granulation tissue are

[illegible] apart from ulceration which is evidently a secondary process. That the organism under discussion is the cause of this deposit cannot be disputed on the lines of Wright's description. It is difficult to conceive of the occurrence of a capsule of the red cell in such a position, and we most probably have to deal with a granulomatous lesion in which the new parasite is the causative agent. It is premature to discuss the relation of the systemic infection by the parasite to the case of granulomatous type. Much depends upon the relation of the disease to the small ulcers and papules described in this paper.

The morphology of the parasite.

I must now ask [illegible] attention to the processes, noted by all observers, of two chromatin masses in the bodies. I showed that the arrangement of these and the structure of the parasite in general is remarkably constant, and that a scheme of the parasite could be drawn which would exhibit when viewed from different planes the most varied, and all the appearances shown by the parasite in films. The "rod" described by some authors as joining the large and small nucleus is a projection or ridge of protoplasm left between two vacuoles. In a consideration of the bodies, especially of the larger forms, I have since made out a further detail of structure. There is a very distinct dark-stained line, starting from the projection or rod of the small chromatin mass and ending as a rule abruptly at a point on the large mass. Lines can be drawn at right angles to this projection, showing that the arrangement is possible to make out most [illegible] of the two hypothetical solves (Pl. II. fig. 1).

In the best stained films I observed the appearance of the bodies of a distinct cuticle. Round the chromatin masses in the stouter parasites and the bodies as a rule [illegible] (Pl. II. figs. 2).

In some films, especially those in which the bodies appear as *pseudo-cysts*, the bodies appear as protoplasmic masses only, and the appearance of a cuticle is absent. The bodies without visible capsule were apparently those of the host. There appears some doubt then as to the resistant power of the apparent capsule, and the appearance may be deceptive. As the bodies appeared not to be piroplasma, and as from their number, their size and their morphology they appeared to resemble somewhat the spores of monocystis, I have made many attempts to cause protrusion of the filament but without success. Blood from the spleen containing the bodies was mixed with ether, strong and weak acids and ammonia. Films were then made, thoroughly washed first in alcohol and then in water, and stained by Romanowski's method. Films made from blood containing the bodies

were placed, without fixing, in 1 per cent. salt solution, and the cover-glass sealed with vaseline. In a few hours the bodies were found to be readily recognisable, and even after the lapse of three weeks, when examined, no appearance of flagella was detected.

The matrix.—Laveran, Ross, Marchiafava and Celli describe a substance in which many of the bodies are embedded. In films made from blood drawn from the spleen during life this "matrix" is easily seen. In films made *post-mortem* it is not to be made out. The appearances are not quite the same in every case, and the matrix may be seen in one or other of the following forms.

(1) As irregular, fragmented and ragged masses. It may be a small and very faint structure, often just visible, or it may be of considerable size. Large masses of this nature, many times larger than a blood corpuscle, may often be seen containing only a single body (Pl. II, figs. 3 and 4).

(2) As well-defined globular masses, smaller than, the same size as, or larger than, a red corpuscle. The substance is as a rule finely reticular. It may be like ground-glass in appearance. Identical bodies can be seen quite free from included parasites, and budding from large cells of a definite leucocytic nature (Pl. II, fig. 5).

(3) Occasionally a faintly staining very *hyaline* substance, small in amount, may be seen in which from 5 to 10 bodies are embedded. The bodies often lie in vacuoles slightly larger than themselves. The amount of matrix in this case is always very small, and it is never found projecting beyond the included bodies.

As regards the appearances described above, there can be no doubt that (1) and (2) are the fragmented and budded cytoplasm of cells of endothelial nature. The undoubted presence in some cases of pigment, either material or derived from the skin and identical with pigment seen in the cells in sections, definitely demonstrates their nature.

The third type, which is much rarer than the others, appears possibly to be residual matter left over from the separation of the multiple division forms described first by Laveran. In the first report I figured bodies having a clear circular outline and containing three or more large, and an equal number of small, chromatin masses, but in which no division into individual bodies could be made out. In sections of the spleen I have been able occasionally to see such forms. They contain as a rule about six large and six small masses. The large masses are arranged peripherally and the small masses centrally. Such forms are enclosed in the cytoplasm of cells as in the case of the bodies ordinarily seen.

Infection of other hosts than man.—I have not succeeded in producing infection by inoculation of the bodies into animals. Monkeys and rabbits were used, and blood containing the bodies was injected subcutaneously into the peritoneum and into the blood stream (intraventricular injection). Up to three weeks after intravascular injection and six weeks after injection into the tissues

of the preparations. No fresh blood films produced and no bodies were found in the spleen of a normal animal.

Comparison of the conditions found *post-mortem* in infection by the new parasite, and in Trypanosome infection.

The organism used for comparison was a highly pathogenic trypanosome which occurs naturally in the pariah dogs of Madras. This trypanosome causes death in dogs in from 6 to 7 weeks after inoculation, and in rats in from 8 to 10 days. The symptoms caused were emaciation, weakness, slight œdema of the hinder parts, scanty skin and distinct paresis of the hind legs. In advanced stages of the disease the trypanosomes were abundant in the peripheral blood.

In animals some hours dead, the trypanosomes were found in the large veins and were readily seen on the field of the microscope. They showed marked contraction. The power of staining of the trypanosome was not in most cases recognisable. The large and small chromatin nucleus and centrosome were apparently unaffected, and the former showed a remarkable resemblance to the chromatin network of the organism in the *peripheral blood*. Numbers of flagellates were everywhere to be seen. They, however, did not stain very deeply and in some examples were scarcely recognisable. Nothing like the definite outline of the new parasite was met with, and the protoplasm was evidently superficial only.

In spleens of dogs and rats dead or killed on the point of death as a result of trypanosome infection, the trypanosomes were found in the blood of the splenic pulp. Though the trypanosomes were abundant in the peripheral blood they were rare in the spleen pulp of the case of dogs and of rats. It was difficult to find trypanosomes included in the spleen cells. Where found, such bodies showed a homogeneous protoplasm which did not in any way resemble those of the new parasite, and were quite unlike the latter in every way. In some cases both the blepharo and nucleus were well to be recognised. In no case was the outline, so characteristic of the new parasite, seen. The large vacuolated cells, seen in infection with the latter, were absent. In none of the animals examined did there appear to be any sign of forms in the spleen. Sections of the spleen showed occasional double chromatin masses in cell protoplasm, but the appearances were otherwise quite unlike those seen in infection with the new parasite. The storing of enormous numbers of the new parasite in an apparently unaltered state in the splenic cells is one of the most remarkable features of infection by these bodies. In trypanosome infection of the dog such a phenomenon does not appear to take place, and from the paucity of included forms one is led to conclude that phagocytosis is not great or that intracellular digestion is rapid.

In a dog strongly infected with *piroplasma canis* many of the leucocytes

contained included forms. Some of the forms showed double chromatin and one had, as in the case of included trypanosomes, a slight resemblance to the oval parasite. In spite of many examinations nothing resembling the segmenting forms was encountered. Contrary to the state of affairs in the case of the oval parasite, intracellular digestion appeared to be in progress and many bodies showed *disorganised and scattered chromatin.*

Conclusions.

1. The bodies described by Wright in tropical ulcer are indistinguishable from those found in cases of enlarged spleen in Madras. In both cases the bodies are for the most part included in the cytoplasm of cells of endothelial nature. In tropical ulcer the bodies appear to be giving rise to a tissue of a distinctly granulomatous type.

2. In so-called malarial cachexia of India the bodies are very numerous in the spleen, liver and bone-marrow. They are in considerable numbers, in some cases at least, in the lungs and testes. In the kidney they do not appear to be in large numbers. In the above named viscera they may occur in leucocytes, but for the most part they are seen in cells of endothelial nature, especially in large cells crowded with the bodies (macrophages).

3. Bodies may be present in large numbers in the granulation tissue associated with ulceration of the large intestine in cases of so-called malarial cachexia.

4. Bodies may generally be found in small numbers in the granulation tissue of small and larger ulcers, and in ulcerated papules on the skin of advanced cases of so-called malarial cachexia. In this case, as in the testis, the bodies are found for the most part lying singly in endothelial cells of the finest capillaries.

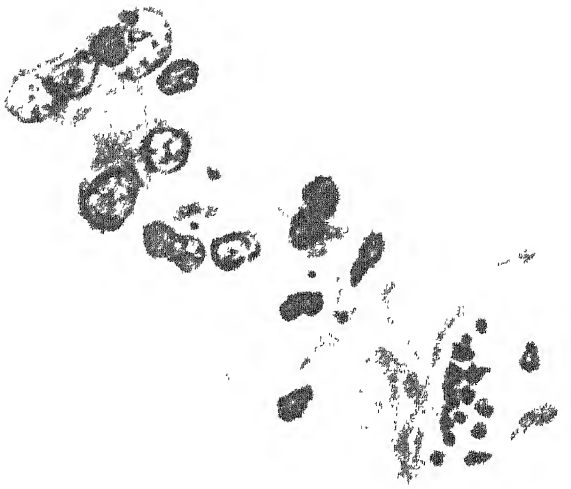
5. Bodies were found in a lymphatic gland which drained a skin lesion containing the bodies. They were not found in a lymphatic gland draining only normal skin.

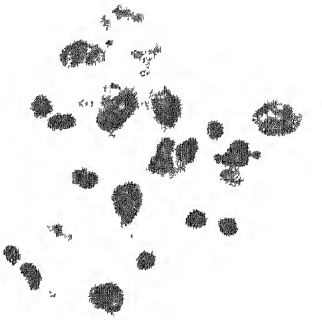
6. Bodies may be found in leucocytes in the peripheral blood. I have not seen unmistakeable forms in red cells either in peripheral or splenic blood.

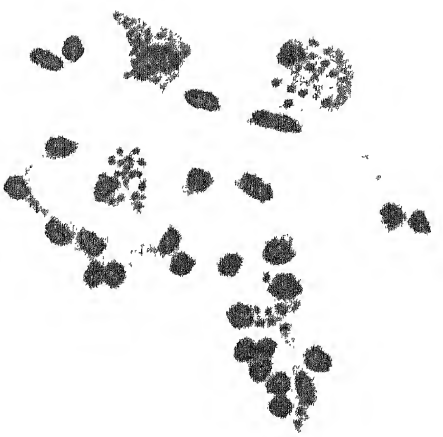
7. The vast majority of the bodies lie in the cytoplasm of endothelium cells. The cachexia is essentially an infection of the vascular endothelium and resembles in many ways a chronic septicæmia.

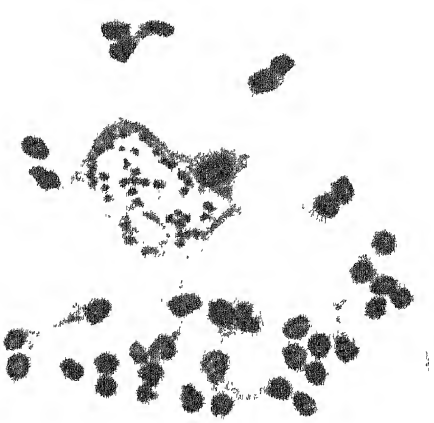
8. The process of infection appears to be as follows. Bodies are taken up by, or invade, endothelium cells in the visceral and certain other capillaries, *e.g.* granulation tissue. The endothelium cells increase in size and become more and more distended with the parasites (macrophages). The cells finally undergo necrosis and appear as mere bags filled with large numbers of the parasites. Eventually such cells would appear to rupture and the contained bodies, which exhibit no trace of intracellular digestion, are thus set free to be taken up again by cells.

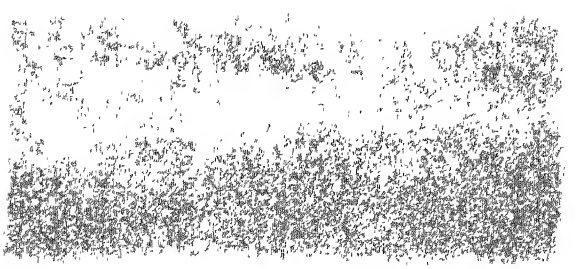
Explanation of Plates

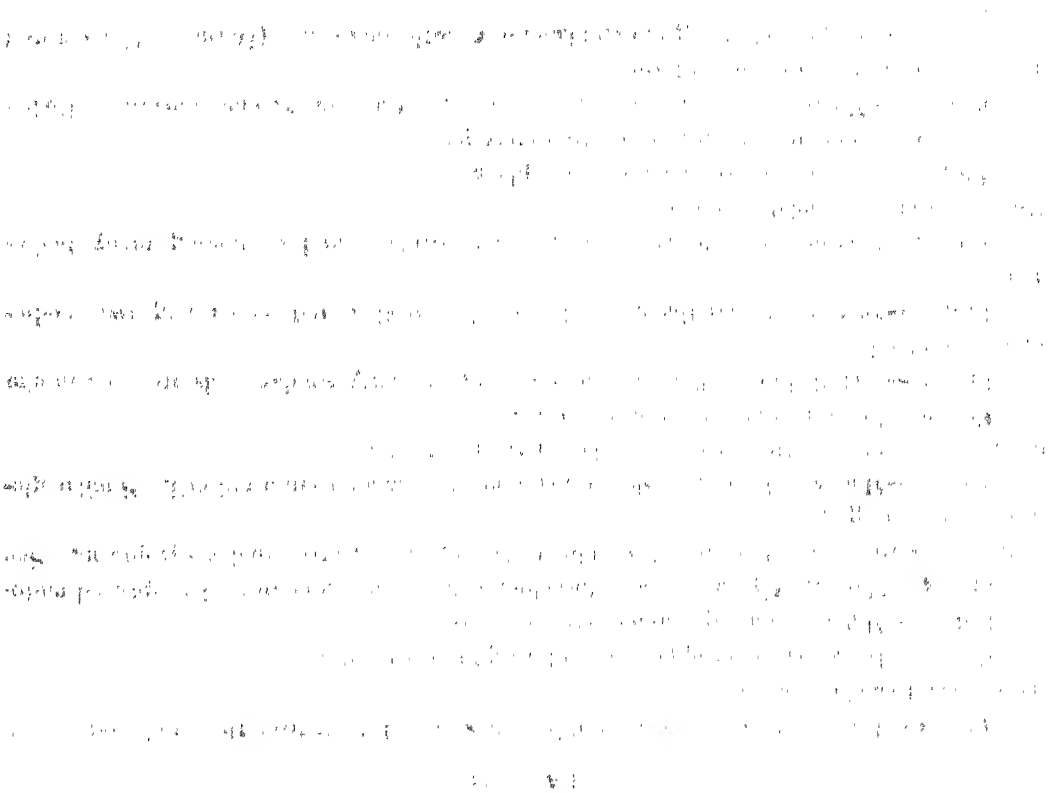


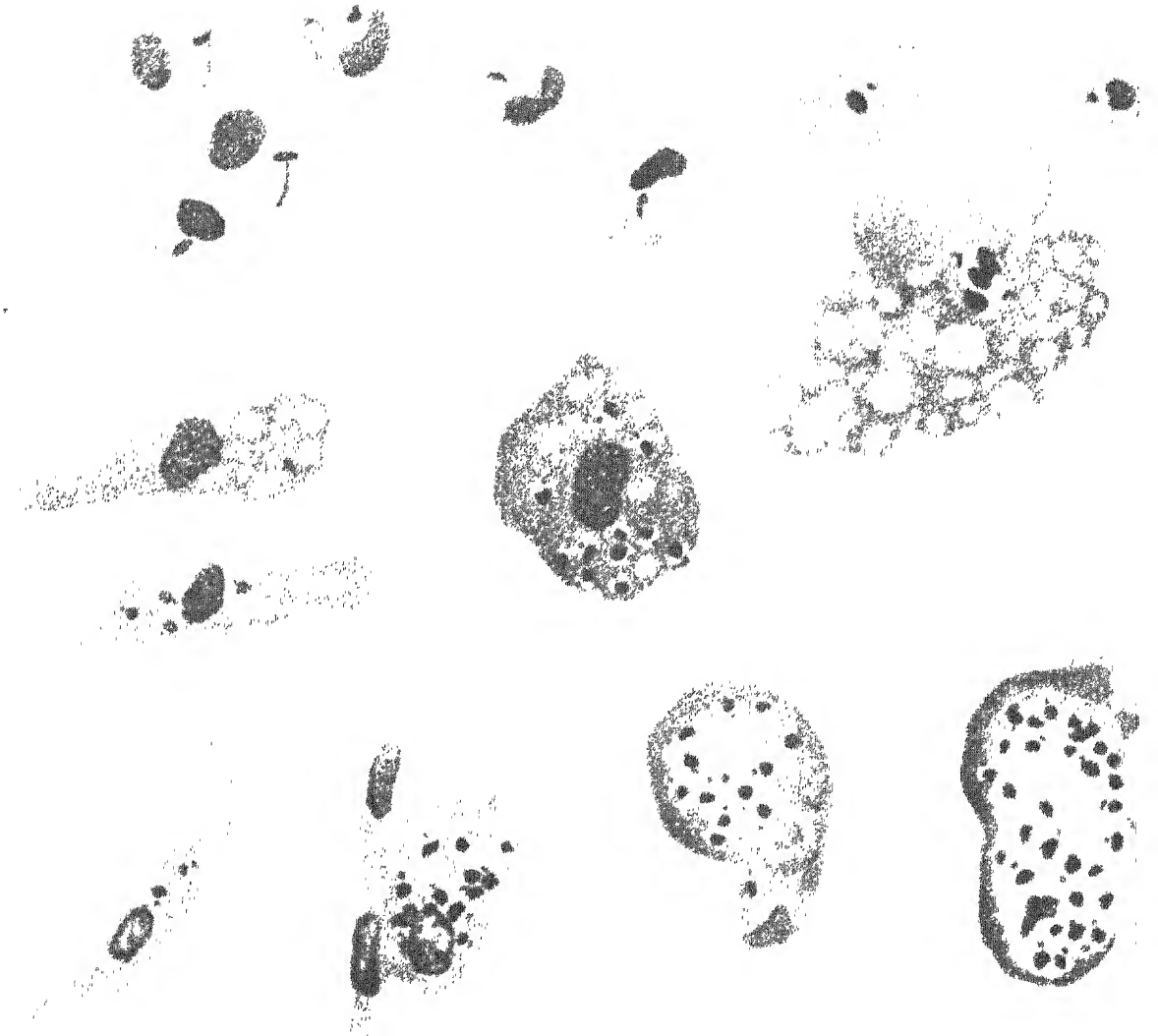


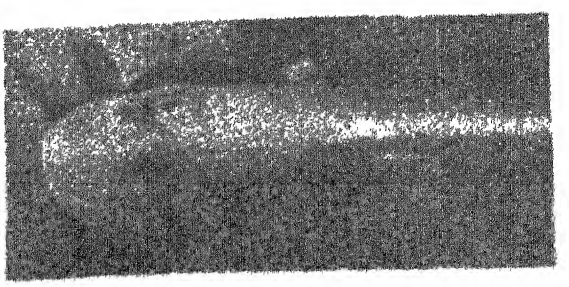


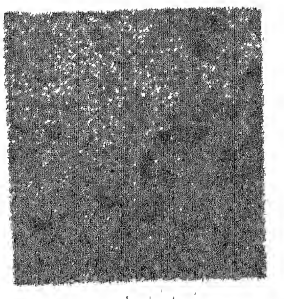












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3 Lancet and Medical Gazette [illegible] (CXXVIII p. 167 4th January 1886)

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7 [illegible] Water Supply Report

No. II.

W SERIES.)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

A PARASITE FOUND IN PERSONS SUFFERING FROM ENLARGEMENT OF THE SPLEEN IN INDIA, SECOND REPORT

BY

LIEUT. S. R. CHRISTOPHERS, M.B., I.M.S.

(On special duty)

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA.

CALCUTTA:

OFFICE OF THE SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA.

1904.